



Southern California Edison Company

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February 24, 1995

Emmanuel K. Mensah

California EPA, Department
of Toxic Substances Control
Region 1
10151 Croydon Way
Sacramento, CA 95827

**SUBJECT: Soil Column Biotreatability Study Work Plan
By The Center For Environmental Microbiology, Inc.
SCE Visalia Pole Yard**

In order to develop design parameters for the engineered bioremediation system at the Visalia Pole Yard, SCE has contracted with the Center for Environmental Microbiology to conduct a 6-month comprehensive column study on site soils impacted with pole-treating chemicals. The column study objective is to determine the degradation rates of key pole-treating chemicals under various environmental conditions. Degradation rates will be compared between various engineered variables and a static control for both the vadose and saturated zones. An additional comparison will be made between disturbed and undisturbed soil columns, to better understand the real degradation rates we are likely to experience during site remediation.

The two main study components are: 1) acquisition of representative site soils, and; 2) the comprehensive soil column study.

The acquisition of representative site soils will be conducted utilizing hollow-stem auger drilling and continuous sampling techniques. The three target zones for soil acquisition are: 1) the vadose zone silts between 30-35 feet (30-foot silt) and 50-60 feet (shallow aquitard); 2) the saturated sands/gravels of the intermediate aquifer (90-100 feet), and; 3) the saturated silts of the intermediate aquitard (100-110 feet). Borings will be located in areas known to contain high concentrations of pole-treating chemicals, based on the previous site characterization. Following sample acquisition, the boreholes will be abandoned utilizing a tremied cement/bentonite slurry backfill.

Following collection, the soil samples will be delivered directly to on-site personnel from the Center for Environmental Microbiology, who will then prepare and transport them to their facility.

The soil column biotreatability study will be conducted in accordance with the attached proposal, prepared by the Center for Environmental Microbiology, Inc.

If you have any questions regarding this study, please call me at (818) 302-2216, or Randy Weidner at (818) 302-4033.

A handwritten signature in black ink that reads "G M Becker". The letters are cursive and somewhat stylized, with a long horizontal stroke at the end of the word "Becker".

George M. Becker
Senior Environmental Specialist

Attachment

cc:

Richard Procunier, USEPA Region 9
T. C. Sciarrotta, SCE
R. S. Weidner, SCE

CENTER FOR ENVIRONMENTAL MICROBIOLOGY, INC.

1660 CHICAGO AVE., SUITE M-2 • RIVERSIDE, CA 92507 • (909) 788-0808 • FAX (909) 788-1691

December 9, 1994

Dr. Ahmed Elseewi
Southern California Edison
P.O. Box 800
2244 Walnut Grove Ave.
Rosemead, CA 91770

Dear Ahmed:

Enclosed please find the revised proposal entitled, "Bioremediation of Polycyclic Aromatic Hydrocarbons and Pentachlorophenol at the SCE Visalia Poleyard." On a separate sheet, I have provided the cost of this proposal. With our thorough discussion on the experimental design, I hope that this proposal is now finalized and we can begin the project as soon as possible.

Thank you for your consideration.

Sincerely,

Bill

W. T. Frankenberger, Jr.
President/CEO and Lab Director

**A Proposal submitted to
Southern California Edison**

**BIOREMEDIATION OF POLYCYCLIC AROMATIC
HYDROCARBONS AND PENTACHLOROPHENOL AT THE
SCE VISALIA POLEYARD**

by

**W. T. Frankenberger, Jr.
Center for Environmental Microbiology, Inc.
1660 Chicago, Suite M-2
Riverside, CA 92507**

November 10, 1994

**Title: Bioremediation of Polycyclic Aromatic
Hydrocarbons and Pentachlorophenol at the
SCE Visalia Poleyard**

Research Personnel: W.T. Frankenberger, Jr., Principal Investigator

Duration of Project: 6 months

Project Summary:

A treatability study is proposed to assess the feasibility of bioremediation of polycyclic aromatic hydrocarbons (PAHs) and pentachlorophenol (PCP) within the subsoil of the SCE Visalia poleyard. Both PAH and PCP are found in the unsaturated and saturated zones in two different matrices, a sand and silt lens. Soil columns will be constructed to simulate an *in situ* treatment to promote the bioremediation of PAHs and PCP. Eighteen disturbed soil columns will be constructed consisting of 6 unsaturated and 6 saturated silt columns and 6 saturated sand columns. The saturated treatments will consist of: i) a control to simulate natural conditions in which the dissolved O₂ (D.O.) content will be maintained at 1 mg L⁻¹, ii) air injection (~8 mg D.O. L⁻¹), and iii) air injection (~8 mg D.O. L⁻¹) plus nutrients (N and P) to maximize bioremediation. The unsaturated treatments will consist of i) a static control, ii) air injection (0.5 to 1.0 air void volumes day⁻¹) and iii) air injection (0.5 to 1.0 air void volumes day⁻¹) plus nutrients (N and P) to simulate bioventing. This experiment will be conducted with duplicate replications. Air will be pumped into the columns with an oil-less air compressor. The nutrients added will consist of (NH₄)₂SO₄ as a nitrogen source and K₂HPO₄ as the phosphorus source. Nutrients (N and P) will be injected into the top of the columns and allowed to percolate by gravity flow. The columns will be maintained at 60°F in a cold room. At specific time intervals (4 weeks) per treatment, subsamples will be removed from the sampling ports of the columns and analyzed for benzo[a]pyrene and PCP. Other parameters to be monitored will include oxygen content, redox potential (E_h), CO₂ levels and the nutrient status throughout the soil matrix.

In addition, 6 undisturbed soil columns (2 saturated sand, 2 saturated silt and 2 unsaturated silt columns) will be obtained to account for the heterogeneity of the two different matrices and i) incubated under field conditions to represent "intrinsic bioremediation" or ii) treated with nutrients (N and P) and injected with air to represent "engineered bioremediation." The same parameters will be monitored as described with the disturbed soil columns including benzo[a]pyrene, PCP, O₂, CO₂, E_h and nutrient status (N and P). The proposed study will identify ecological factors that could influence the biodegradation rates of PAHs and PCP in the subsoil.

PROJECT DESCRIPTION

Description of Problem, Importance and Scientific Basis

Polycyclic Aromatic Hydrocarbons

By definition, polycyclic aromatic hydrocarbons (PAHs) consist of 2 or more fused benzene rings either arranged linearly or clustered. The number and position of the various rings can significantly alter their bioavailability. The aqueous solubilities of PAHs vary with molecular weight. The following table displays the diversity of PAHs and their solubilities.

Compound	Formula	Solubility (mg/L)*
Napthalene	C ₁₀ H ₈	31.7
Acenaphthlene	C ₁₂ H ₁₀	3.93
Fluorene	C ₁₃ H ₁₀	1.98
Anthracene	C ₁₄ H ₁₀	0.073
Pyrene	C ₁₆ H ₁₄	0.135
Chrysene	C ₁₈ H ₁₂	0.0020
Coronene	C ₂₄ H ₁₂	0.00014

* Source Mackay and Shiu, 1977.

Polycyclic aromatic hydrocarbons are hydrophobic and tend to sorb to organic particulates and clay particles as a function of pH, CEC and clay content (Means et al., 1980). This sorption significantly decreases their availability for degradation. The distribution of PAHs in the aqueous phase is often the rate limiting step for microbially mediated degradation. Solubility tends to be one of the major factors giving rise to the observation that PAHs consisting of 2 and 3 rings are more readily degraded than those of higher molecular weights. Since the higher molecular weight PAHs are more persistent and more toxic, interest in increasing their availability for microbial degradation has recently been investigated.

In general, PAHs containing two or three aromatic rings are readily degraded. PAHs containing four or more aromatic rings are more recalcitrant. The degradation of PAHs presents an

additional concern since some are oxidized to intermediates that are chemically carcinogenic. As with benzene, the oxidation of di- and tri-ring PAHs such as naphthalene, anthracene and phenanthrene, involve the formation of a dihydrodiol intermediate. A considerable amount of information exists on the microbial metabolism of anthracene and phenanthrene. *Pseudomonas putida* and *Berijerinckia* sp. strain B-836 oxidize anthracene in the 1,2-positions to *cis*-1,2-dihydroxy-1,2-dihydroanthracene (Gibson and Subramanian, 1984). Intermediate products of *cis*-3,4-dihydrophenanthrene and *cis*-1,2-dihydroxy-1,2-dihydrophenanthrene are produced by these organisms. Two pathways for phenanthrene are known (Gibson and Subramanian, 1984). *Cumminghamella elegans* oxidizes phenanthrene at the 1,2 and 3,4 positions to form *trans*-1,2- and *trans*-3,4-dihydrodiols. *Cumminghamella elegans* also oxidizes anthracene to *trans*-1,2-dihydroxy-1,2-dihydroanthracene. The growth rates of bacteria and fungi on naphthalene, phenanthrene, and anthracene appear to be related to the water solubilities of these aromatic hydrocarbons. The lower solubility of anthracene ($73 \mu\text{g L}^{-1}$) limits its bioavailability.

Less information is available on the bacterial degradation of PAHs that contain more than three rings. Microbial oxidation, however, of compounds such as fluorene, fluoranthene, pyrene, benzo[a]pyrene, benzo[a]anthracene and dibenzo[a]anthracene does occur, but at relatively slow rates. The initial metabolites formed are *cis*-dihydrodiols (Cerniglia et al 1986).

The importance of cometabolism for the higher molecular weight PAHs has been demonstrated. Recent studies have indicated that degradation of the higher molecular weight PAHs are enhanced when a lower molecular weight PAH is present and is the primary substrate (Cerniglia et al 1984). In the presence of either naphthalene or phenol-naphthalene, cometabolism of pyrene, 1,2-benzanthracene, 3,4-benzpyrene and 1,2,5,6-dibenzanthracene by a mixed culture of *Flavobacterium* sp. and *Pseudomonas* sp. has been demonstrated. Environmental studies have indicated that the rate limiting step for aerobic metabolism of PAHs may be the initial ring oxidation reaction.

Pentachlorophenol

Pentachlorophenol (PCP) was widely used in the United States for wood preservation both for newly cut timber and for slime control in pulp and paper production. PCP is metabolized by a variety of microorganisms. There are a few studies that have identified metabolites arising from pure culture metabolism of PCP. Cultures of *Pseudomonas* spp produce both tetrachlorocatechol and tetrachlorohydroquinone from PCP. These intermediates are metabolized rapidly soon after they are produced. Pentachlorophenol is metabolized by *Arthrobacter* sp. to pentachloroanisole (Neilson et al., 1983). A bacterium identified as *Microbacterium* sp. which cannot use PCP as a growth substrate methylates PCP to pentachloroanisole. Recently, a saprophytic soil corynebacterium was isolated which utilizes PCP as the sole source of carbon and energy for growth. By measuring $^{14}\text{CO}_2$ evolution, a conversion rate was calculated to be 10 mg PCP mg^{-1} dry cell weight per hour.

The role of fungi in detoxifying PCP has been studied to some degree. Three *Trichoderma* spp. metabolized sodium pentachlorophenate (Na-PCP) within 2 weeks in a malt extract medium as well as on wood treated with Na-PCP (Cserjesi, 1967). Pentachloroanisole was detected in the culture media of *Trichoderma virgatum* after 5 days of incubation at levels corresponding to 10-20% of the starting Na-PCP.

In soil systems, PCP has been shown to be readily degraded. A soil perfusion apparatus using rice paddy soil demonstrated the disappearance of PCP with more than 90% liberation of chloride (Watanabe, 1973). A *Pseudomonas* sp. isolated from an enrichment culture degraded 40 mg L^{-1} PCP in 10 days with complete chloride release. PCP added to a moist garden soil at 150-200 mg L^{-1} soil-water concentration was 25% metabolized after 12 days when the experiment was conducted using outdoor shaded test plants.

Comparison of aerobic and anaerobic metabolism of PCP has shown that aerobic metabolism is much more efficient (Liu et al., 1981). Enrichment cultures established in fermentors fed with 2 mg L^{-1} PCP revealed a half-life of 0.36 days under aerobic conditions and 192 days under anaerobic conditions. Soils treated with 10 mg L^{-1} PCP and incubated for 24 days

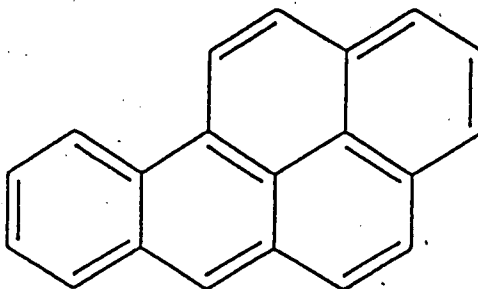
under aerobic conditions revealed considerable loss of ^{14}C -labeled PCP (Murthy et al., 1979). Of 59% total recovered material, 51% was identified as pentachloroanisole. The rate of PCP metabolism in 11 soils was found to be related to the organic matter content of the soil. Degradation products included a mixture of tri- and tetra-chlorophenols.

A major PCP spill on the Mississippi River Gulf outlet left PCP levels as high as 1,600 mg kg^{-1} in the sediment (DeLaune et al., 1983). After 18 months, there was no detectable PCP in the sediment. Studies arising from the spill indicated that the degradation rates increased with increasing sediment redox potential. Maximum degradation occurred at pH 8 (+500 mV).

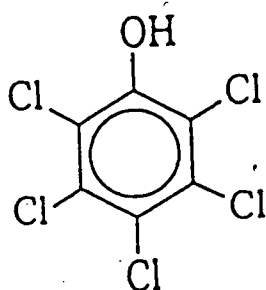
In summary, there is evidence that PCP is attacked by bacteria and fungi, cometabolically or as a sole source of growth with the release of chloride and CO_2 . The pathway involves dechlorination and hydroxylation, either *ortho* or *para* to the phenolic hydroxyl group, forming a catechol or a quinone, respectively. However, the mechanism of this process is not well understood and the enzymes involved have not been isolated. In fungi, methylation has been detected as a predominate metabolic process, but its role in PCP degradation has not been established.

Rationale, Objectives and Experimental Approach

The rationale for the proposed study is to obtain information on possible factors that could influence the biodegradation rates of PAHs and PCP in the saturated and unsaturated subsoil zones of the SCE Visalia Poleyard. Benzo[a]pyrene is one of the more resistant PAHs to microbial degradation. This investigation will focus on benzo(a)pyrene which has the following physical and chemical properties: Henry's Law Constant, $2.4 \times 10^{-6} \text{ atm}\cdot\text{m}^3/\text{mol}$; $\log K_{oc}$, 5.60; $\log K_{ow}$, 5.99; melting point, 178-179°C; solubility in water, approximately 0.0038 mg/L at 25°C; specific density, 1.351 at 20/4°C.



Pentachlorophenol has also been detected in the unsaturated and saturated subsoil of this site. The physical and chemical properties of pentachlorophenol are as follows: Henry's Law Constant, 3.4×10^{-6} atm·m³/mol; log K_{oc} , 2.95; log K_{ow} , 5.01; melting point, 191°C; solubility in water, specific density, 1.978 at 22/4°C; 1.7×10^{-4} mm at 20°C.



The specific objectives of this treatability study are as follows:

1. To determine the degradation rate of benzo[a]pyrene and PCP under both saturated and unsaturated conditions of two matrices (a sand and silt lens) under conditions of "intrinsic" bioremediation.
2. To determine the movement and effectiveness of nutrients in soil columns in stimulating benzo[a]pyrene and PCP degrading activity.
3. To determine the success of enhanced bioremediation upon air injection into the soil columns.

Experimental Design

I. Characterization of the Contaminated Matrices

Physicochemical properties of the contaminated sand and silt will be characterized in terms of pH, inorganic nitrogen ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$), inorganic phosphorus (orthophosphate-P), moisture content and particle size analysis.

The microbiota density within the subsurface will be determined by conducting total heterotrophic microbial counts utilizing nutrient agar and quantifying specific PAH- and PCP-degrading microorganisms in the subsoil collected on site.

Soil Column Study

Soil columns, 3 ft in length x 3 inch (dia.) Plexiglass, will be constructed to simulate an *in situ* treatment to promote bioremediation of PAHs and PCP (Fig. 1). Twelve columns will consist of the disturbed silt lens, while 6 more columns will be made up of sand. The sand and one-half of the silt columns will be subject to saturated conditions, while the other 6 disturbed silt columns will be maintained unsaturated. The saturated treatments will consist of: i) a control to simulate natural conditions in which the dissolved O_2 (D.O.) content will be maintained at 1 mg L^{-1} , ii) air injection ($\sim 8 \text{ mg D.O. L}^{-1}$) and iii) air injection ($\sim 8 \text{ mg D.O. L}^{-1}$) plus nutrients (N and P) to maximize bioremediation. The unsaturated treatments will consist of i) a static control, ii) air injection (0.5 to 1.0 air void volumes day^{-1}) and iii) air injection (0.5 to 1.0 air void volumes day^{-1}) plus nutrients (N and P) to simulate bioventing. Air will be pumped into columns with an oil-less air compressor. The nutrients will consist of $(\text{NH}_4)_2\text{SO}_4$ as a nitrogen (N) source and K_2HPO_4 as the phosphorus (P) source. Nutrients (N and P) will be injected into the top of the columns and allowed to percolate by gravity flow. During incubation, the loss of water in the unsaturated soil columns will be monitored with a moisture meter (Soil Moisture Equipment Corp., Santa Barbara, CA) connected to gypsum blocks. The columns will be maintained at 60°F in a cold room.

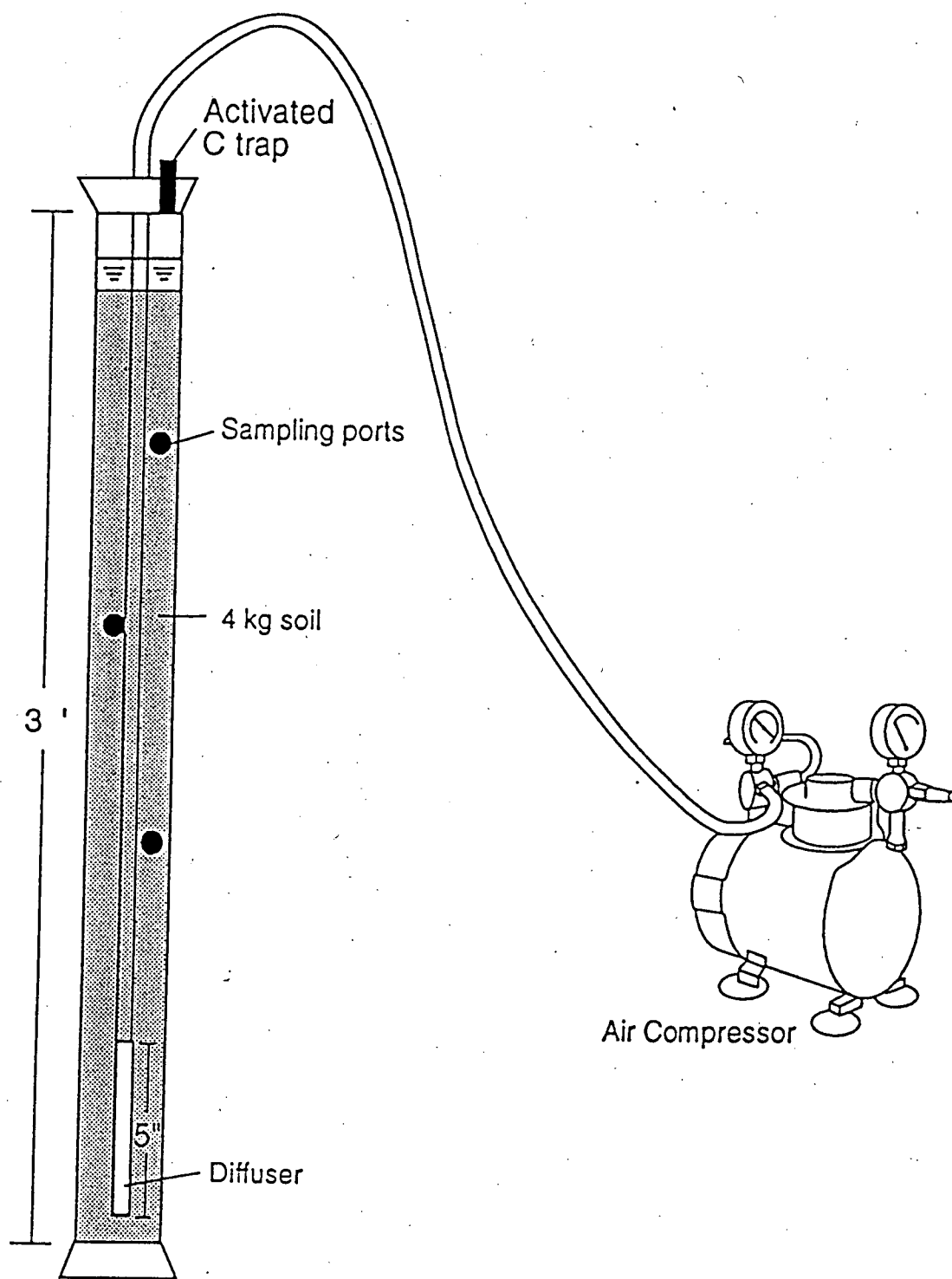


Fig. 1. Experimental illustration of soil columns subject to bioventing/biosparging.

In addition, six 3-ft undisturbed soil columns (2 saturated sand, 2 saturated silt and 2 unsaturated silt) will be obtained to account for the heterogeneity of the two different matrices. These columns will be packed on site to minimize disturbance. They will then be transported to the laboratory and incubated under field conditions, to represent "intrinsic bioremediation" or treated with nutrients (N and P) and injected with air to represent "engineered bioremediation."

At specific time intervals (4 weeks) per treatment, subsamples will be removed from the sampling ports of the columns and analyzed for benzo[a]pyrene and PCP (EPA 8270). Other parameters which will be monitored will include O_2 content, redox potential (E_h), CO_2 levels and the nutrient (N and P) status throughout the soil matrix.

The O_2 content will be monitored with an O_2 electrode based on polarographic O_2 measurements with a built-in Ag/AgCl electrode. This system provides a low O_2 consumption rate, a fast response time, low sensitivity with easy calibration and no stirring required. A readout through a chemical microsensor as a polygraphic amplifier uses a polarization source. This system is ideal for monitoring the pO_2 content under both saturated and unsaturated conditions in intact systems with minimal disturbance.

The redox will be measured with platinum electrodes connected to a millivolt meter.

A micro- CO_2 electrode connected to a portable pH meter will be used to monitor the biological activity in the soil columns. This electrode can measure CO_2 readings of 10^{-2} to 10^{-4} M (440 to 4.4 ppm) with sensitivity of 0.4 mV/mn CO_2 . The response time is <1 min.

The nutrient analysis will include NH_4 -N (EPA 350.1), NO_3 -N (EPA 352.1) and orthophosphate-P (EPA 365.1) content.

Report preparation

After the data is collected with this treatability study, it will be organized and interpreted to provide recommendations for a pilot scale project. Monthly progress reports will be provided with progressive graphs (conc vs. time), raw data and discussion of results. The final report will be completed 3 weeks after the last scheduled day of collecting data.

REFERENCES

- Cerniglia, C.E. 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. In: *Advances in Applied Microbiology*. A. Laskin, Ed., Academic Press, New York, vol. 30, pp.31-71.
- Cerniglia, C.E., D.W. Kelly, J. P. Freeman and D.W. Miller. 1986. Microbial metabolism of pyrene. *Chem. Biol. Interactions*. 57:203-216.
- Cserjesi, A.J. 1967. The adaptation of fungi to pentachlorophenol and its biodegradation. *Can. J. Microbiol.* 13:1243-1249.
- DeLaune, R.D., R.P. Gambrell, and K.S. Reddy. 1983. Fate of pentachlorophenol in estuarine sediment. *Environ. Pollut. (Ser. B)*6:297-308.
- Gibson, D.T. and V. Subramanian. 1984. Microbial Degradation of Aromatic Hydrocarbons. In: *Microbial Degradation of Organic Compounds*. D. T. Gibson, Ed, Marcel Dekker, Inc. New York, vol. 13, pp. 181-252.
- Liu, D., K. Thomson, and W.M.J. Strachan. 1981. Biodegradation of pentachlorophenol in a simulated aquatic environment. *Bull. Environ. Contam. Toxicol.* 26:85-90.
- Means, J.C., S.G. Wood, J.J. Hassett and W.L. Banwart. 1980. Sorption of polynuclear aromatic hydrocarbons by sediments and soils. *Env. Sci. and Tech.* 14:1524-1528.
- Murthy, N.B.K., D.D. Kaufman, and G.F. Fries. 1979. Degradation of pentachlorophenol (PCP) in aerobic and anaerobic soil. *J. Environ. Sci. Health B*14:1-14.
- Nash, R.G., and E.A. Woolson. 1967. Persistence of chlorinated hydrocarbon insecticides in soils. *Science* 157:924-927.
- Watanabe, I. 1973. Isolation of pentachlorophenol decomposing bacteria from soil. *Soil Sci. Plant Nutr.* 19:109-116.

UNDISTURBED SOIL COLUMNS

Parameters to be monitored	Silt		Sand
	Saturated (2)*	Unsaturated (2)**	Saturated (2)
Benzo[a]pyrene	xxxxxxx [†]	xxxxxxx	xxxxxxx
Pentachlorophenol	xxxxxxx	xxxxxxx	xxxxxxx
Oxygen levels	xxxxxxx	xxxxxxx	xxxxxxx
CO ₂ levels	xxxxxxx	xxxxxxx	xxxxxxx
Redox potential (E _h)	xxxxxxx	xxxxxxx	xxxxxxx
NH ₄ -N and NO ₃ -N	xxxxxxx	xxxxxxx	xxxxxxx
Orthophosphate-P	xxxxxxx	xxxxxxx	xxxxxxx

* (2) represents two soil columns in which one will be incubated under field conditions (maintained at 1 mg L⁻¹ of dissolved O₂) to simulate "intrinsic bioremediation" while the other will be treated with nutrients (N and P) and injected with air (to maximize the dissolved O₂ content) to represent "engineered bioremediation."

** (2) represents two soil columns in which one will be incubated under static conditions to simulate intrinsic "bioremediation" while the other will be treated with nutrients (N and P) and injected with air to represent "bioventing."

[†] Represents time of sampling (0, 1, 2, 3, 4, 5, 6 months)

QUALIFICATIONS

Dr. William T. Frankenberger, Jr.

Academic Positions:

1990-present Professor of Soil Microbiology & Biochemistry, UCR
 1986-1990 Associate Professor of Soil Microbiology & Biochemistry, UCR
 1981-1986 Assistant Professor of Soil Microbiology & Biochemistry, UCR

Education:

Ph.D. 1980 Soil Microbiology & Biochemistry, Iowa State University
 M.S. 1977 Soil Microbiology & Biochemistry, Iowa State University
 B.S. 1974 Biology, Kansas State Teachers College

Honors & Awards:

1981 Soil Science Society of America Emil Truog Award
 1987 American Society of Agronomy Visiting Scientist Award
 1991 Fellow, Soil Science Society of America
 1991 Fellow, The American Institute of Chemists
 1992 Fellow, American Society of Agronomy
 1993 Fellow, American Association for the Advancement of Science
 1993 Fellow, American Academy of Microbiology
 1993 Invited Speaker, Gordon Research Conference,
 Applied & Environmental Microbiology, July 1993
 1994 President Elect of Soil Microbiology & Biochemistry Division,
 Soil Science Society of America

Professional and Honorary Societies:

American Society for Microbiology; American Academy of Microbiology; American Association for the Advancement of Science; American Chemical Society; American Institute of Chemists; American Society of Agronomy; Soil Science Society of America; International Society of Soil Science; Sigma Xi; Gamma Sigma Delta; Council for Agricultural Science and Technology; Plant Growth Regulator Society of America; National Water Well Association

Research Interests:

Bioremediation of Hazardous Chemicals; Microbial Transformations of Trace Elements

Teaching:

Soil Science 111, Soil Microbiology and Biochemistry
 Environmental Science 155, Principles and Applications of Bioremediation

Contracts and Grants: (1985-94) \$2,458,472

U.S. Dept. of the Interior, Bureau of Reclamation; California Water Resources Control Board/U.S. EPA; CSRS/U.S. Dept. of Agriculture, Water Quality Program; Kearney Foundation of Soil Science; U.C. Salinity/Drainage Task Force; USDA Cooperative State Research Award; USDA Forest Service; American Society of Agronomy; TMC Properties/U.S. EPA; Kelco (Merck); Roche Vitamins and Fine Chemicals; U.C. Integrated Pest Management Program; Pacific Gas and Electric Co.; California Avocado Development Organization; Imperial County Whitefly Management Program

Publications:

Books: 2; Reviews and Book Chapters: 20; Technical Journal Articles: 110;
 Technical Reports: 40

Plenary Lectures and Presentations: 73

Invited Lectures: 72

Patents: 1